Mapping and Comparison of Quantitative Trait Loci for Oleic Acid Seed Content in Two Segregating Soybean Populations

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ABSTRACT

Soybean [*Glycine max* (L.) Merr.] produces 29.4% of the world's edible vegetable oil. An important determinant of the nutritional value and the oxidative stability of soybean oil is the oleic acid content. Elevation of the oleate content levels leads to the improvement of soybean oil quality. However, our knowledge of the genetic factors underlying oleate variation in soybean seeds remains incomplete, hampering the use of marker-assisted selection in soybean breeding programs. We used a whole-genome scan approach to identify oleate quantitative trait loci (QTLs) in a soybean population segregating for oleic acid content and a cognate segregating population to confirm oleate QTL. A novel oleate QTL with moderate effects was revealed on linkage group F in the proximity of the simple sequence repeat marker sat_309, which was confirmed in both populations across all environments tested. Furthermore, this study verified the existence of an oleate QTL with moderate effects in the proximity of *FAD2-1B* isoform on linkage group I, which interacted epistatically with the oleate QTL on linkage group F. Oleate QTLs with moderate effects were also detected on linkage groups A2 and N only in one of the populations under study. Minor QTLs on linkage groups E, L, A1, and D2 confirmed previous mapping studies for oleate content in soybean.

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Abbreviations: BLUP, best linear unbiased predictor; CIM, composite interval mapping; FAD, fatty acid desaturase; LOD, likelihood of odds; MIM, multiple interval mapping; MQM, multiple-QTL model mapping; QTL, quantitative trait locus; RFLPs, restriction fragment length polymorphisms; SSR, simple sequence repeat.

Soybean [*Glycine max* (L.) Merr.] is the leading oilseed crop in the United States in terms of gross vegetable oil production and economic importance (Wilcox, 2004). To retain its competitive position in domestic and global markets, the oilseed industry has to abide by the guidelines of the United States Food and Drug Administration, which now require lower levels of *trans* isomers found in food products (Wilson et al., 2002). *Trans* isomers are formed during hydrogenation, a process that enhances the long-term oxidative stability of soybean oil. The need for oil hydrogenation can be diminished by reducing the levels of the polyunsaturated linoleic and linolenic acids, which are susceptible to oxidation, through the concomitant elevation of the levels of the oxidatively stable monounsaturated oleic acid (Wilson, 2004). The high-oleate and low-linolenate traits have been successfully incorporated into soybean germplasm through conventional breeding (Wilson et al., 1981; Burton et al., 1989; Burton et al., 2006) and genetic engineering (Kinney, 1995; Kinney and Knowlton, 1998; Buhr et al., 2002). However, our knowledge on

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the genetic factors that cause the observed variation in oleate and linoleate contents remains incomplete.

Previous studies reported quantitative trait loci (QTLs) controlling the unsaturated fatty acid content in soybean. Currently, 19 QTLs are designated in SoyBase (http://soybeanbreederstoolbox.org/; verified 15 Jan. 2009); these include six oleate QTLs on linkage groups A1, B2, and E, six linoleate QTLs on linkage groups A1, B1, E, K, and L, and seven linolenate QTLs on linkage groups E, K, and L (Diers and Shoemaker, 1992). These QTLs were detected in an $F_{2:3}$ population derived from the cross of the normal-oleate/normal-linolenate *Glycine max* line A81356022 with the normal-oleate/high-linolenate *G. soja* line PI 468916 using restriction fragment length polymorphisms (RFLPs), isozymes, and morphological markers. Confidence intervals of oleate QTLs coincided with those of linoleate QTLs and had additive effects in the opposite direction (Diers and Shoemaker, 1992). In a recent study, six oleate QTLs were mapped on linkage groups A1, D2, G, and L in two soybean population of $F_{2:3}$ lines derived from the crosses of the normal-oleate experimental lines G99-G725 or G99-G3438 with the high-oleate line N00-3350, a single plant selection of N98-4445A (Monteros et al., 2008). Several minor QTLs have also been detected for oleate (linkage groups D1b, L, and E), linoleate (linkage groups F, L, and E) and linolenate (linkage groups F, L, E, and G) traits. The QTLs on linkage groups L and E conditioned both mono- and polyunsaturated fatty acid traits and, as expected from the flux through the fatty acid biosynthesis pathway, they had opposite effects (Hyten et al., 2004; Panthee et al., 2006).

Besides the QTLs that control oleate, linoleate, and linolenate content, the isoforms of the *GmFAD2* (Heppard et al., 1996; Schlueter et al., 2007) and *GmFAD3* (Anai et al., 2005; Bilyeu et al., 2003) genes, encoding the microsomal ω-6 and ω-3 desaturase enzymes, respectively, are also implicated in the genetic control of the unsaturated fatty acid content. Evidence for the existence of oleate QTLs with only minor effects in the proximity of *FAD2-1B* on linkage group I and possibly *FAD2-2B* on linkage group L were presented by Bachlava et al. (2008a). On the contrary, *FAD3A* cosegregated with the *fan* locus, a major linolenate gene localized on linkage group B2 (Brummer et al., 1995; Byrum et al., 1997; Bilyeu et al., 2003; Spencer et al., 2004).

The lack of major oleate QTLs cosegregating with the *FAD2-1A*, *FAD2-1B,* and *FAD2-2B* isoforms (Bachlava et al., 2008a) suggested that a whole-genome scan approach is necessary to shed light on the genetic factors that cause the observed genotypic variation for oleate content in populations in which this trait is segregating. Thus, the objectives of this study were to (i) map QTLs for oleic acid seed content in two soybean populations segregating for the trait; (ii) estimate their genetic effects and the amount of oleate variation they explain; and (iii) report the epistatic interactions among the identified QTLs and the QTL × environment interactions.

MATERIALS AND METHODS

Population Development and Experimental Design

Two soybean populations, denoted as FAF and FAS, were developed by single seed descent (Brim, 1966). The FAF population consisted of 118 F_5–derived lines from the cross of N97-3363-3 × PI 423893 and the FAS population consisted of 231 F_3–derived lines from the cross of N98-4445A × PI 423893. N98-4445A is a high-oleate (563.1 g kg^{-1}) low-linoleate line, which was developed by the USDA-ARS in Raleigh, NC (Burton et al., 2006). N98-4445A originated as a plant selection from the cross N94-2473 × (N93-2007-4 × N92-3907) and is a sister line of N97-3363-3. PI 423893 is a mid-oleate (305.8 g kg^{-1}) plant introduction with unknown genetic background (USDA-ARS National Plant Germplasm System, http://www.ars-grin.gov/npgs/searchgrin.html). N98-4445A, N97-3363-3, and PI 423893 had determinate growth habit. Apart from oleate genes, the FAF and FAS populations were also segregating for the reduced linolenate *fan* (PI 123440) allele.

The FAF population was planted in a sets-within-replications experimental design (Hallauer and Miranda, 1988) with two replications in each location, as described by Bachlava et al. (2008a, 2008b). The FAF population was grown at Clinton and Kinston, NC, in 2005 and at Clinton, Kinston, Clayton, and Plymouth, NC, in 2006. Each experimental line of the FAF population was planted in a four-row plot, with the exception of Clayton in 2006, where each line was planted in a one-row plot. The FAS population was planted in five-seed hills in a sets-within-replications design with three replications at Clayton in 2006 and 2007, as described by Bachlava et al. (2008a, 2008b).

Phenotypic Evaluation and Statistical Analysis

Maturity date was recorded at the R8 reproductive stage (Fehr and Caviness, 1977) as days after planting for all the environments where the FAF population was grown, and for the FAS population in 2006. Late planting of the FAS population in 2007 resulted in insufficient variation of the experimental lines' maturity dates. Mature soybean seeds were harvested mechanically from the two middle rows of each experimental plot of the FAF population or from each one-row plot at Clayton in 2006. Approximately 10 g of seeds were subsampled from the seeds harvested from each four-row plot for the evaluation of fatty acid composition. For the FAS population 5 g were subsampled from the seeds harvested from each hill. Fatty acid composition was evaluated by gas liquid chromatography using a Model 6890 GC (Agilent Technologies, Inc., Wilmington, DE), as described by Burkey et al. (2007).

Statistical analyses of the phenotypic data for the FAF and FAS populations, which were both planted in sets-within-replications designs, were conducted using Proc MIXED in SAS 9.1 (SAS, 2004). Environments, sets, replications, lines, and their interactions were considered random effects. Best linear

unbiased predictors (BLUPs) were obtained for maturity and fatty acid traits. For each experimental line of the FAF and FAS population, BLUPs were estimated as the sum of the intercept and the random effect (Littell et al., 1996). The phenotypic data of the FAF population from Clinton, NC, in 2005 were discarded due to excess missing data and greater error variance compared to the other environments. BLUPs were derived separately for each environment and combined across all environments for both the FAF and FAS populations. To test whether accounting for maturity effects influences the QTLs detected herein, BLUPs that included maturity date as a covariate in the mixed model were also obtained. BLUPs accounting for maturity derived in each environment separately were estimated as the sum of the intercept, the random effect, and the average maturity date multiplied by the slope of the covariate, for each experimental line of the FAF and FAS populations.

Genotypic Evaluation and Linkage Mapping

For each of the FAF experimental lines, genomic DNA was extracted from leaf tissue collected from approximately ten plants using the Gentra PureGene DNA purification kit (Gentra Systems, Minneapolis, MN). For each of the FAS experimental lines, genomic DNA was isolated from leaf tissue collected from all plants of each hill using a modified CTAB protocol described by Keim et al. (1988).

A total of 164 polymorphic simple sequence repeat (SSR) markers covering the 20 linkage groups of the soybean genome, according to the consensus linkage map (Cregan et al,. 1999; Song et al., 2004), were genotyped for the FAF population. The FAS population was genotyped with 88 SSR markers flanking the genomic regions where QTLs were detected for the FAF population or previous studies and in the proximity of the isoforms of *FAD2-1* and *FAD2-2* genes. The polymerase chain reactions were performed in a 384-well PTC-200 Peltier Thermal Cycler (MJ Research, Waltham, MA). Thermocycling conditions were 95°C for 2 min and 38 cycles of 92°C for 1 min, 49°C for 1 min, and 68°C for 1 min 30 s. The amplification products were resolved on 4% SFR agarose gels (Amresco, Solon, OH) with ethidium bromide staining in 1x Tris-Borate-EDTA buffer, or 6.5% polyacrylamide gels in a LICOR 4300 DNA Analysis System (LICOR Biosciences, Lincoln, NE) using M13-tailed unmodified primers and IRD-700 or IRD-800 labeled M13 oligonucleotides.

Isoform- and allele-specific markers were previously developed for *FAD2-1A*, *FAD2-1B,* and *FAD2-2B*. *FAD2-1A* was genotyped with an allele specific primer extension (ASPE) assay in the FAF population and with a single base extension (SBE) assay in the FAS population. *FAD2-1B* and *FAD2-2B* were genotyped in both populations using cleaved amplified polymorphic sequence (CAPS) markers. The marker development and mapping of *FAD2-1A*, *FAD2-1B,* and *FAD2-2B* on the soybean genome were described by Bachlava et al. (2008a). Also, the FAF population was genotyped with the *FAD3A* specific marker developed by Camacho-Roger (2006).

Linkage analysis of the FAF population was conducted with MAPMAKER/EXP 3.0 (Lander et al., 1987) according to Cardinal et al. (2001), using a minimum likelihood of odds (LOD) score of 3.0 and a maximum Kosambi distance of 40 cM. The heterozygote genotypes were discarded in order for the F_5–derived lines to be analyzed as recombinant inbred lines. Linkage analysis of the FAS population was conducted with JoinMap 3.0 (Van Ooijen and Voorrips, 2001), since analyzing the F_3–derived lines as F_2 lines with MAPMAKER/EXP 3.0 resulted in the distortion of map distances. For the JoinMap 3.0 analysis, the linkage map was constructed using the Kosambi's mapping function and linkage was declared with maximum recombination frequency of 0.4 and minimum LOD score of 3.0.

QTL Mapping

QTL analysis was conducted with WinQTL Cartographer version 2.5 (Wang et al., 2005) for maturity and fatty acid traits across environments and in each environment separately using the BLUPs derived for each experimental line of the FAF and FAS population. QTLs were identified with multiple interval mapping (MIM) (Kao et al., 1999) using a whole-genome scan approach in FAF, whereas the FAS population was used for QTL confirmation. Initially, CIM procedure (Zeng, 1993) was implemented with a minimum LOD threshold of 2.5 to identify QTLs that were inputted in a starting model in MIM. The "forward and backward regression" method was used for cofactor selection and the genome scans were conducted with window size of 10 cM and walk speed of 2 cM. The CIM results for each trait and environment were inputted in the MIM procedure using the "scan through CIM analysis" option. The models were refined using the "optimize positions," "search for new QTLs," and "test existing QTLs" options, for main effects, in both populations, and epistatic interactions, in the FAS population. MIM models with the minimum Bayesian information criterion (BIC) were chosen and "search for new QTLs" was conducted once, since additional searches resulted in the detection of QTLs with very small effects. Final models were confirmed using the "MIM forward selection" option. The estimates of the QTL effects and the proportion of the variation explained from each QTL of the final model were outputted using the "summary" option. For the FAF population, only additive effects were fitted in the model due to the advanced inbreeding generation.

The output of the QTL analysis for the FAF population was verified with PLABQTL version 1.2 (Utz and Melchinger, 2006), which utilizes a multiple regression procedure in contrast to the maximum likelihood approach of WinQTL Cartographer. CIM analysis in PLABQTL was performed using the "cov SEL" statement for cofactor selection and LOD threshold derived from 1000 permutations at the 0.05 significance level. Akaike's information criterion (AIC) was used for the selection of the best CIM model. Due to limitations in the number of characters for the datasets analyzed by PLABQTL, supplementary QTL analysis for the FAS population was conducted with MapQTL 5 (Van Ooijen, 2004) using the default settings. Markers selected during the interval mapping (IM) were used as cofactors for the multiple-QTL model (MQM) mapping. QTL analyses discussed below refer to MIM conducted with QTL Cartographer unless indicated otherwise. QTL × environment interactions were tested with Proc GLM in SAS 9.1 for the markers that mapped in the proximity of detected QTLs in FAF and FAS. ESTIMATE and CONTRAST statements determined the contribution of each environment to the interaction.

RESULTS AND DISCUSSION

Linkage Mapping

Two mapping populations, denoted as FAF and FAS, were developed by single-seed descent (Brim, 1966) and consisted of 118 F_5–derived lines from the cross of N97-3363-3 × PI 423893 and of 231 F_3–derived lines from the cross of N98-4445A × PI 423893, respectively. For the FAF population, a linkage map was constructed with 151 SSR markers covering the 20 linkage groups of the soybean genome. Segregation distortion was observed for six markers at the 0.0025 significance level; while 13 of the 164 genotyped SSR markers could not be mapped. The length of the linkage map was 1646 cM, which suggests an adequate coverage of the genome considering that the consensus linkage map is 2536 cM in length (Song et al., 2004). The order of the majority of SSR markers coincided with the consensus genetic map of soybean (Cregan et al., 1999; Song et al., 2004). The map contained 13 linkage gaps as a result of the lack of polymorphic SSR markers. In addition, the linkage gaps may be attributed to the fact that recombination estimates obtained from a population of modest size have large standard errors and, therefore, it is statistically more difficult to reject the null hypothesis of no linkage. The average distance between SSR markers was approximately 14.6 cM. The FAS population was genotyped with molecular markers flanking previously identified oleate QTLs, either in the FAF population or previous studies, and markers in the proximity of *FAD2-1A*, *FAD2-1B,* and *FAD2-2B* isoforms. Eight of the 88 genotyped SSR markers remained unmapped. The length of the genomic region covered was 964 cM and the SSR markers mapped on average 17.8 cM apart.

QTL Mapping Overview

Six oleate QTLs were identified for each of the FAF and FAS populations, explaining 54.9 and 57.4% of the genotypic variation for oleic acid content, respectively (Tables 1 and 2). The QTL with the largest effect was mapped on linkage group F and explained 16.5 and 18.3% of the respective oleate variation in the FAF and FAS populations (Tables 1 and 2). However, due to the modest population sizes, the genetic effects are most likely overestimated (Beavis, 1994). The high oleate allele of the QTL on linkage group F was inherited from PI 423893 in both the FAF and FAS populations, whereas the high oleate alleles were inherited from N97-3363-3 for the remaining QTLs detected in the FAF population. Of the six QTLs identified for FAS population, three QTLs inherited the high oleate allele from N98-4445A and the remaining three QTLs from PI 423893. With the exception of the oleate QTL on linkage group F, the QTLs identified in the FAF and FAS populations explained less than 12% of oleate variation, which emphasizes the quantitative nature of oleate trait that is controlled by several minor QTLs. It should be noted that oleate content ranged from 253.3 to 457.3 g kg^{-1} in FAS and 301.2 to 551.4 g kg^{-1} in FAF (Bachlava et al., 2008b); therefore, both populations would be expected to segregate for at least some major oleate QTLs.

The comparison of the oleate QTLs identified across environments initially suggested a lack of congruency between the mapping results of the FAF and FAS populations (Tables 1 and 2). However, comparison of the oleate QTLs identified in each environment tested for both populations (Tables 3 and 4) revealed that the QTLs mapped in the Clayton 2006 environment for the FAF population (linkage groups D2 and M) were also identified for the FAS population, which was grown only at the Clayton 2006 and Clayton 2007 environments. Moreover, QTLs that were identified across environments for the FAF population, but were not detected at Clayton 2006 (linkage groups N and E), were also absent from the FAS population (Tables 3 and 4). These results suggested environmental influence on oleate traits, albeit oleate heritabilities on a plot and an entry mean basis were previously reported higher that 0.75 for both the FAF and FAS populations (Bachlava et al., 2008b). With the exception of the QTLs on linkage groups D2, M, N, and E, oleate QTLs mapped in FAF and FAS were consistent across the majority of environments tested in each population (Tables 3 and 4) and their estimated effects were similar in direction and magnitude (data not shown).

Apart from the environmental effects on oleate traits, the discrepancies observed between the QTLs identified for the FAF and FAS populations may be attributable to the limitations of QTL mapping as an approach for identifying the genetic factors controlling quantitative traits. The drawbacks of QTL mapping include the lack of congruency among mapped QTLs due to the effects of different environments, genetic backgrounds, number and type of marker loci, level of inbreeding, and data analysis techniques. Also, the power of QTL detection is dependent on the size of the mapping populations and the heritability of the traits (Beavis, 1994; Bernardo, 2002). In this study, the two mapping populations were derived from crosses of sister maternal lines with the same paternal plant introduction; thus, they are expected to segregate for common oleate genes. Oleate heritabilities were also high for both the FAF and FAS populations (Bachlava et al., 2008b). Therefore, the identification of different oleate QTLs for the FAF and FAS populations may be related to differences in the level of inbreeding and the size of the two populations. Another possible interpretation may be the lower power of detection of genetic factors with minor effects, such as the QTLs controlling oleate traits, using the present QTL mapping approaches, which may result in inconsistent identification of QTLs across different environments.

Five linoleate QTLs were detected for each of the FAF and FAS populations, explaining 60.1 and 46.4% of the genotypic variation for linoleate content, respectively (Tables 1 and 2). All linoleate QTLs corresponded to QTLs identified for oleate content for each of the two mapping populations and their estimated effects had opposite directions, as expected from the strong negative genetic correlations between the two traits that was higher than –0.90 in both populations (Bachlava et al., 2008b). Although current QTL mapping analysis alone cannot determine whether the cause of the correlated effects for the two traits is due to linkage or pleiotropy, our understanding of the flux through the fatty acid biosynthesis pathway favors the latter.

In contrast, only one linolenate QTL was simultaneously mapped for oleate and linoleate traits in the FAF population, and the linolenate shared no common QTLs with oleate or linoleate traits in the FAS population, as expected from the low genetic correlations between linolenate and oleate (–0.422 in FAF and –0.292 in FAS) and between linolenate and linoleate (0.222 in FAF and –0.084 in FAS) contents (Bachlava et al., 2008b). A major linolenate QTL was identified on linkage group B2 that cosegregated with the *FAD3A* gene, which has been previously mapped to the *fan* locus (Brummer et al., 1995; Byrum et al., 1997; Bilyeu et al., 2003; Spencer et al., 2004). As shown in Tables 1 and 2, the linolenate QTL on linkage group B2 explained 35.9% of the observed variation in the FAF population and 23.2% in the FAS population. Significant QTL × environment interactions were detected for the linolenate QTL on linkage group B2 (specifically the *FAD3A* marker) in the FAF population. The additive effects of *FAD3A,* estimated using Proc GLM in SAS 9.1, varied from 14.9 g kg^{-1} in the Kinston 2005 environment to 22.4 g kg^{-1} in the Clayton 2006 environment.

Comparisons of the number and the estimated effects of QTLs identified using MIM with those identified using the CIM analysis in WinQTL Cartographer (data not shown) illustrate the increased power of QTL detection of MIM procedure. However, CIM analysis was only used to identify QTLs that will be later tested in MIM models. MIM not only has more statistical power for QTL mapping, but has also improved precision for the estimation of QTL positions and meets CIM limitations, such as the estimation of epistatic effects and the joint contribution of multiple linked QTL to the phenotypic variance (Zeng, 1993; Wang et al., 2005). Moreover, the QTLs detected using the CIM procedure of PLABQTL for the FAF population and the MQM procedure of MapQTL for FAS coincided with the majority of the QTLs mapped using the CIM analysis of WinQTL Cartographer. For the FAF population, the CIM procedure of PLABQTL identified the oleate QTLs on linkage groups F, E, N, and A1; while, for the FAS population, the MQM procedure of MapQTL identified the oleate QTLs on linkage groups F, G, and I (data not shown).

Table 1. Quantitative trait loci (QTLs) for oleate, linoleate, linolenate, and maturity date detected with multiple interval mapping (MIM) in the FAF population (N97–3363–3 × PI 423893) based on best linear unbiased predictors (BLUPs) derived across environments. The linkage group (LG) and the marker interval where each QTL was mapped, as well as its size, the distance of the QTL peak from the most nearby marker (underlined), the additive effect of the QTL, and the proportion of variation explained by each QTL (R^2) is reported.

Trait	LG	Marker interval	Interval size	Distance from nearby marker	Additive Effect†	R^2
			cM	cM	g kg^{-1}/DAP	%
Oleate	N‡	satt022– sat_304	14.00	4.01	21.041	11.2
	A2‡	sat_294– satt333	13.20	5.19	21.827	11.6
	F‡	satt206– sat_309	23.20	7.19	–25.512	16.5
	I	sat_324– sat_299	14.60	5.10	16.726	7.4
	A1	satt050– satt619	18.90	0.01	12.646	3.7
	E	sat_273– satt651	16.10	0.01	15.243	4.5
					Total R^2 § (%)	54.9
Linoleate	N‡	satt022– sat_304	14.00	2.01	–15.525	9.4
	A2	satt333– satt233	31.40	10.01	–24.897	20.6
	F‡	satt206– sat_309	23.20	6.00	22.061	16.7
	I	sat_324– sat_299	14.60	6.60	–16.502	10.1
	A1	satt225– sat_217	4.10	0.09	–10.026	3.3
					Total R^2 § (%)	60.1
Linolenate	L‡	satt495– sat_301	9.40	5.39	2.602	6.1
	A2‡	satt409– sat_294	5.20	1.20	–2.336	5.6
	D1b	satt634– sat_423	45.60	11.59	–3.338	10.6
	B2	satt687– *FAD3A*	17.30	3.29	–6.334	35.9
	A1	satt684– sat_368	9.80	1.79	–2.231	5.1
					Total R^2 § (%)	63.3
Maturity	B2‡	*FAD3A*– satt066	17.00	2.99	1.518	13.5
	F‡	sat_309– sat_133	3.30	1.29	–1.767	15.7
	L	satt495– sat_301	9.40	4.00	–1.080	5.3
	L‡	*FAD2–2B*– satt561	14.70	4.01	1.362	10.7
	I	satt571– satt496	18.90	6.01	–1.134	4.9
	B1	sat_270– satt509	9.90	0.01	0.959	4.8
					Total R^2 § (%)	54.9

†Additive effect of the QTL was estimated with MIM analysis as the difference of the homozygous N97-3363-3 and PI 423893, measured in g kg^{-1} for fatty acids and days after planting (DAP) for maturity. Positive additive effects indicate that the N97-3363-3 allele increases the value of the trait.

‡QTL detected with composite interval mapping (CIM) analysis using minimum likelihood of odds (LOD) threshold of 2.5.

§Total variation explained when all QTLs are simultaneously fitted in the MIM model.

Comparison of QTLs Identified for the FAF and FAS Populations

The QTL for oleate and linoleate traits on linkage group F was detected for both mapping populations of this study

Table 2. Quantitative trait loci (QTLs) for oleate, linoleate, linolenate, and maturity date detected with multiple interval mapping (MIM) in the FAS population (N98-4445A × PI 423893) based on best linear unbiased predictors (BLUPs) derived across environments. The linkage group (LG) and the marker interval where each QTL was mapped, as well as its size, the distance of the QTL peak from the most nearby marker (underlined), the additive and/or dominance effect of the QTL, and the proportion of variation explained by each QTL (R^2) is reported.

Trait	LG	Marker interval	Interval size	Distance from nearby marker	Effect (A/D)†	R^2
			cM	cM	g kg^{-1}/DAP	%
Oleate	M	satt220– sat_226	27.76	11.76	38.802 (D)	11.7
	G‡	satt472– satt288	19.80	1.96	11.152 (A)	5.0
	D2	sat_300– satt301	7.90	0.08	10.084 (A)	2.7
	O‡	sat_108– satt153	11.99	5.98	–8.401 (A)/ –17.726 (D)	2.8/2.7
	I‡	satt354– *FAD2–1B*	4.91	2.12	–16.387 (A)	9.8
	F‡	sat_133– sat_309	8.94	0.10	–22.912 (A)	18.3
	I x F #				7.630 (A x A)	1.7
					Total R^2 § (%)	57.4
Linoleate	G‡	satt472– satt288	19.80	0.01	–9.232 (A)	4.0
	D2‡	satt002– satt669	19.84	6.01	–10.818 (A)	5.9
	O	*FAD2–1A*– sat_108	24.02	6.01	7.582 (A)/ 15.096 (D)	3.1/3.0
	I‡	satt354– *FAD2–1B*	4.91	0.90	10.745 (A)	7.1
	F‡	sat_133– sat_309	8.94	0.10	20.199 (A)	20.9
	I x F #				–6.263 (A x A)	1.6
					Total R^2 § (%)	46.4
Linolenate	B2‡	sat_355– satt066	24.33	0.32	–6.074 (A)	23.2
	J	satt244– sat_395	28.10	10.09	12.144 (D)	16.3
					Total R^2 § (%)	39.7
Maturity¶	M‡	sat_226- sat_256	16.11	2.01	1.097 (A)	6.6
	I‡	*FAD2–1B*– sat_268	5.96	0.01	–1.038 (A)	6.5
	O‡	satt478– satt420	25.84	1.83	–1.027 (A)/ –1.656 (D)	7.1/2.3
	L‡	satt006– sat_113	11.83	5.32	2.285 (A)	26.1
	B2	sat_355– satt066	24.33	8.32	0.666 (A)/ 2.269 (D)	2.9/4.7
	M x L #				–1.073 (A x A)	1.9
					Total R^2 § (%)	59.2

†Additive effect of the QTL was estimated with MIM analysis as the difference of the homozygous N98-4445A and PI 423893 genotypes and dominance effect was estimated as the difference of the heterozygous genotypes from the mean of the homozygous N98-4445A and PI 423893 genotypes, measured in g kg^{-1} for fatty acids and days after planting (DAP) for maturity. Positive additive effects indicate that the N98-4445A allele increases the value of the trait. Additive and dominance effects of the QTL were both included in the table when each effect explained more than 1.5% of the variation.

‡QTL detected with composite interval mapping (CIM) analysis using minimum likelihood of odds (LOD) threshold 2.5.

§Total variation explained when all QTLs were simultaneously fitted in the MIM model.

¶QTL analysis for maturity date was conducted with BLUPs derived from a single environment (Clayton 2006).

#Epistatic interactions between the designated QTLs.

and was stable across all environments tested (Tables 3 and 4). This oleate QTL explained 16.5 and 18.3% of the genotypic variation in the FAF and FAS populations, respectively (Tables 1 and 2). It should be noted that the locations where the oleate QTL mapped for the FAF and FAS populations are adjacent but not identical marker intervals. This inconsistency is probably due to the different SSR markers genotyped for the two populations (satt206 was not polymorphic in the FAS population) and the difference between the two linkage maps as a consequence of the different population sizes and generation advancement of the two populations.

A maturity QTL was also mapped in the proximity of the oleate QTL on linkage group F in the FAF population (Table 1), which explained approximately 15.7% of the genotypic variation for maturity date and was identified in three of the five environments tested (data not shown). In the FAF population, maturity ranged from 103.6 to 122.7 days after planting across environments (Bachlava et al., 2008b). However, QTL mapping for oleate and linoleate traits using BLUPs accounting for maturity effects revealed that the QTL on linkage group F is not an artifact of maturity differences among the experimental lines that could lead to different temperatures during the period of oil deposition and, therefore, different fatty acid profiles. Also, no QTL for maturity was identified on linkage group F in the FAS population, where the QTLs for oleate and linolenate traits were consistently detected in both environments tested (Table 4). Significant QTL × environment interactions were detected for the QTL on linkage group F (SSR marker sat_133) only in the FAS population for both oleate and linoleate contents. The ranges of the additive effects of the sat_133 marker for oleate and linoleate, which were estimated with Proc GLM in SAS 9.1, were –32.1 to –57.2 g kg^{-1} and 28.9 to 50.0 g kg^{-1} in the Clayton 2006 and Clayton 2007 environments, respectively.

QTL Cosegregating with the *FAD2-1B* Isoform

The peak of the QTL identified on linkage group I for the FAS population, which explained 9.8% of oleate variation and 7.1% of linoleate variation across environments (Table 2), was located less than 3 cM from the *FAD2-1B* gene, previously mapped in the interval between satt354 and sat_268 (Bachlava et al., 2008a). These results suggested that allelic variants of *FAD2-1B* cosegregated with the oleate and linoleate QTLs detected for the FAS population. The location and the effect of the QTLs were consistent in the Clayton 2006 and Clayton 2007 environments (Table 4). The high oleate allele was inherited from PI 423893 and resulted in the increase of oleate content by 16.387 g kg^{-1} across environments

Table 3. Comparison of quantitative trait loci (QTLs) for oleate detected with multiple interval mapping (MIM) based on best linear unbiased predictors (BLUPs) derived across environments with oleate QTLs detected based on BLUPs derived separately for each environment where the FAF population (N97-3363-3 × PI 423893) was tested. The marker interval, where each QTL mapped, and the marker closer to the peak of the QTL (underlined) are reported.

LG†	Across‡	Kinston 2005	Kinston 2006	Clayton 2006	Clinton 2006	Plymouth 2006
N	satt022-sat_304	satt022-sat_304	satt022-sat_304		satt022- sat_304	
A2	sat_294-satt333	sat_294-satt333	sat_294- satt333	satt333-satt233	sat_294- satt333	sat_294-satt333
F	satt206- sat_309	satt206-sat_309	satt206-sat_309	satt206-sat_309	satt206-sat_309	satt206-sat_309
I	sat_324- sat_299	sat_418-sat_324	sat_418-sat_324	sat_324- sat_299		sat_324- sat_299
A1§	satt050-satt619	satt050- satt619	satt300-satt050	satt225- sat_217	satt225- sat_217 satt050- satt619	satt225- sat_217
E	sat_273- satt651	sat_273- satt651	sat_273- satt651		sat_273- satt651	
				D2¶ (sat_300- satt301) M¶ (sat_226- satt220)	J¶ (sat_224- sat_395)	M¶ (sat_226- satt220)

†Linkage group.

‡QTLs detected using BLUPs derived across environments.

§Two distinct QTL positions were reported on linkage group A1 in different environments, but both QTLs were simultaneously fitted only in one of the environments tested (Clinton 2006).

¶QTLs that were not detected using BLUPs derived across environments.

(Table 2). The results of this study are in agreement with Bachlava et al. (2008a), where single factor analysis (SFA) revealed a minor QTL near *FAD2-1B*.

The oleate and linoleate QTL also coincided with a maturity QTL of smaller effect; however, the QTL for both traits was detected when the analysis accounted for maturity effects in the Clayton 2006 environment, where maturity ranged from 92.6 to 110.9 days after planting (Bachlava et al., 2008b). In the Clayton 2007 environment, the lack of variation in maturity among the experimental lines was further evidence that the QTL on linkage group I explained the observed variation in oleate and linoleate contents. 'Additive × additive' epistatic interactions with minor effects were identified between the oleate and linoleate QTLs on linkage groups I and F and were consistent across environments (Table 4). Selection of the QTL on linkage group F led to a further increase in oleate content by 7.630 g kg^{-1} across environments (Table 2).

Table 4. Comparison of quantitative trait loci (QTLs) for oleate detected with multiple interval mapping (MIM) based on best linear unbiased predictors (BLUPs) derived across environments with oleate QTLs detected based on BLUPs derived separately for each environment where the FAS population (N98-4445A × PI 423893) was tested. The marker interval, where each QTL mapped, and the marker closer to the peak of the QTL (underlined) are reported.

LG†	Across‡	Clayton 2006	Clayton 2007
M	satt220–sat_226	satt220–sat_226	
G	satt472–satt288	satt472–satt288	satt472–satt288
D2§	sat_300–satt301	sat_300–satt301	satt135–satt002
O	sat_108–satt153	sat_108–satt153	*FAD2-1A*–sat_108
I	satt354–*FAD2-1B*	satt354–*FAD2-1B*	satt354–*FAD2-1B*
F	sat_133–sat_309	sat_133–sat_309	sat_133–sat_309
L¶			satt006–sat_113
	I × F#	I × F#	I × F# G × L#

†Linkage group.

‡QTLs detected using BLUPs derived across environments.

§Two distinct QTL positions were reported on linkage group D2 in 2006 and 2007.

¶QTL that was not detected using BLUPs derived across environments.

#'Additive × additive' epistatic effects.

Comparison of QTLs across Mapping Studies

Four QTLs reported here verified previous mapping studies for the unsaturated fatty acid content in soybean. Comparisons among QTLs identified from different studies were conducted according to Cardinal et al. (2001), with the assumption that QTLs mapped less than 20 cM apart were common. Thus, the minor oleate QTL detected on linkage group E for the FAF population (Table 1) confirmed previously reported results of Panthee et al. (2006) that identified QTLs for oleate, linoleate, and linolenate in the proximity of SSR marker satt263. This oleate QTL explained 4.5% of oleate genotypic variation across environments in the FAF population (Table 1) and varied from 3.2 to 5.0% in separate environments (data not shown). The high oleate allele was inherited from N97-3363-3 and resulted in an increase of 15.243 g kg^{-1} in oleic acid content (Table 1). The minor oleate QTL on linkage group E was not detected for the FAS population, but the absence of the QTL from the Clayton 2006 environment of the FAF population (Table 4) implied that it may be due to the environmental effect of this location on the QTL.

Another QTL that validated previous studies is that on linkage group L, which was only detected for oleate and linoleate traits in the Clayton 2007 environment of FAS population and mapped in the interval of the SSR markers satt006 and sat_113 (Table 4). This QTL on linkage group L coincided with an oleate QTL reported by Monteros et al. (2008) and QTLs for oleate, linoleate, and linolenate traits detected by Hyten et al. (2004) mapped within the

same marker interval. The N98-4445A allele in this study and the N00-3350 allele in the study by Monteros et al. (2008) increased oleate content. The minor additive and dominance genetic effects of this locus explained less that 4% of oleate and linoleate variation in the FAS population (data not shown). A QTL for maturity was also mapped in the same marker interval in the Clayton 2006 environment of the FAS population and across environments for the FAF population. The maturity QTL on linkage group L was previously identified by Orf et al. (1999) near the gene for growth habit *Dt1*. The lack of significant maturity differences across the experimental lines, due to the late planting of the FAS population in 2007, implies that the QTL on linkage group L, which was only detected in that environment, actually explains the observed variation for oleate content.

On linkage group A1, oleate and linoleate QTLs were identified in the proximity of the RFLP markers A082_1, A104_1, and A170_1 by Diers and Shoemaker (1992). Monteros et al. (2008) confirmed the existence of a minor QTL around the SSR marker satt200. In our study, two distinct oleate QTLs were identified for the FAF population on linkage group A1. The oleate QTL located in the interval of satt225 and sat_217, which was detected for the Clayton 2006, Clinton 2006, and Plymouth 2006 environments, coincided with the previously reported QTL on linkage group A1 (Table 3). This QTL explained 2.3, 3.9, and 3.5% of oleate variation in the three environments, respectively (data not shown). The N97-3363-3 allele increased oleate content, which is in agreement with the study by Monteros et al. (2008) where the high oleate allele was inherited from N00-3350, a single plant selection of N98-4445A.

The results of this study also confirm the presence of a minor oleate QTL on linkage group D2, which was identified by Monteros et al. (2008). The QTL on linkage group D2 was mapped in the interval of the SSR markers sat_300 and satt301 for the Clayton 2006 environments in the FAF and FAS populations (Tables 3 and 4), and explained 4.3 and 5.0% of the observed variation for oleate content in the two populations, respectively. The high oleate allele of the QTL on linkage group D2 was inherited from the N97-3363-3 and N98-4445A lines in the FAF and FAS populations, respectively, and the N00-3350 line in the study by Monteros et al. (2008).

CONCLUSIONS

This study revealed a novel QTL for oleic acid content with moderate effects located on linkage group F in the proximity of the SSR marker sat_309, which was confirmed by both the FAF and FAS populations across all environments tested. Since the high oleate allele was inherited from PI 423893 in both populations, the QTL on linkage group F can contribute to the further increase of oleate content of the high-oleate lines N97-3363-3 and N98-4445A. Moreover, oleate QTLs with moderate effects were reported on linkage groups A2 and N. These QTLs were not validated by the FAS population possibly due to environmental effects. These effects were evident for the QTL on linkage group N, which could not be identified in the Clayton 2006 environment. Minor oleate QTLs were also detected on linkage groups E, L, A1, and D2, confirming previous mapping studies for oleic acid content in soybean (Diers and Shoemaker, 1992; Hyten et al., 2004; Monteros et al., 2008; Panthee et al., 2006).

Furthermore, this study verified the existence of an oleate QTL with moderate effects in the proximity of *FAD2-1B* isoform on linkage group I, previously reported by Bachlava et al. (2008a). Although the oleate QTL, which was detected only in the FAS population, coincided with a maturity QTL on linkage group I, further analysis suggested that it is not an artifact of the variation in maturity dates among the experimental lines. Interestingly, 'additive × additive' epistatic interactions were observed between the oleate QTL on linkage group F, which exhibited the largest additive effects in both mapping populations in this study, and the oleate QTL near *FAD2-1B* isoform on linkage group I. Further investigation is required to verify the cosegregation of *FAD2-1B* with the oleate QTL on linkage group I in different genetic backgrounds and to determine its stability across environments.

In conclusion, an overall view of the QTL mapping studies to date, shows that oleic acid content is a complex quantitative trait controlled by several minor QTLs that are not always stable across environments and mapping populations. Therefore, marker-assisted selection for oleate content is not likely to be effective unless a few loci with relatively large effects that are consistent across breeding populations are identified (Holland, 2004).

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